

儿茶素诱导的拟南芥根细胞膜脂变化^{*}

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摘要: 儿茶素是一种可以短时间内杀死植物细胞的植物毒素, 由于具有强的植物毒性, 儿茶素是开发除草剂的理想化合物, 它可以诱导植物根系统的死亡。为了研究植物根细胞膜脂对化学胁迫的响应规律, 我们运用高通量的脂类组学方法检测了拟南芥根中膜脂分子的组成, 比较了儿茶素处理下拟南芥野生型(WS)及磷脂酶D8缺失突变体(PLD8-KO)根中膜脂分子的组成情况、膜脂含量、双键指数及碳链长度值。结果发现, 儿茶素处理拟南芥根90 min后, 二半乳糖基二酰甘油(DGDG)、单半乳糖基二酰甘油(MGDG)、磷脂酰甘油(PG)、磷脂酰胆碱(PC)及磷脂酰肌醇(PI)的总含量在WS与PLD8-KO植株根中都显著下降, 磷脂酰乙醇胺(PE)和磷脂酰丝氨酸(PS)在WS中下降, 在PLD8-KO中上升。儿茶素处理导致PLD8-KO植株的PC/PE比值显著下降, WS植株PS碳链长度显著增加。上述结果说明儿茶素处理后, 磷脂酶D8缺失突变体膜不稳定增加, PLD8-KO植株对儿茶素胁迫更加敏感。

关键词: 植物毒素; 脂类组学; 儿茶素; 拟南芥

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Profiling of Membrane Lipids of *Arabidopsis* Roots during Catechin Treatment

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Abstract: Catechin is a kind of phytotoxin and can kill plant cells in an hour. It can be developed as herbicide to kill weeds due to its strong phytotoxic activity. The main effect of this chemical is to trigger the death of the root system. To understand the response of root cell membrane lipids to catechin stress, we used the lipidomics approach to study the profiles of *Arabidopsis* root lipids molecules under catechin treatment. The changes of molecular species in membrane lipids, content of membrane lipids, double bond index (DBI) and acyl chain carbon number of the fatty acid were examined in Wild type (WS) and PLD8 deficient mutant (PLD8-KO) during catechin treatment. The results indicated that after 90 min treatment with catechin, the lipid contents of digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), phosphatidylglycerol (PG), phosphatidylcholine (PC), and phosphatidylinositol (PI) decreased both in WS and PLD8-KO roots, lipid contents of phosphatidylethanolamine (PE) and phosphatidylinositol (PS) decreased in WS roots, but increase in PLD8-KO roots, lipid contents of (phosphatidic acid) PA increased at the begin of treatment and declined to the level of control in WS roots. The ratio of the two major lipids in roots, PC and PE, declined significantly in PLD8-KO plants, the acyl carbon number of PS in WS plants

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increased. The results suggested that PLD δ -KO is more sensitive than WS during catechin treatment, and suppression of PLD δ exacerbated membrane damage induced by catechin.

Key words: Phytotoxin; Lipidomics; Catechin; *Arabidopsis*

Abbreviation: electrospray ionization tandem mass spectrometry, ESI-MS/MS; phosphatidylcholine, PC; phosphatidylethanolamine, PE; phosphatidylglycerol, PG; phosphatidic acid, PA; phosphatidylinositol, PI; phosphatidylserine, PS; digalactosyldiacylglycerol, DGDG; monogalactosyldiacylglycerol, MGDG; PLD, phospholipase D

Catechin is a common compound in plants which has antioxidant activity (Lee *et al.*, 2002; Lu *et al.*, 2011; Ma *et al.*, 2003; Meyer *et al.*, 1998; Ono *et al.*, 1998; Su *et al.*, 2002; Vuong *et al.*, 2011; Yang *et al.*, 2003). However, recent researches were focused on the phytotoxic and antibacterial activity of catechin that released from some plants to help them compete with surrounding plants. For example, Inderjit *et al.* (2008) found that catechin can significantly inhibit root growth of *Bambusa* and *Koeleria* seedlings; Bais *et al.* (2003) reported that catechin can inhibit seed germination of 6 kinds of weeds and crops; a study by D'Abrosca *et al.* (2006) displayed that (−)-catechin can inhibit green alga *Selenastrum capricornutum* growth; (+)-catechin from seed coat of *Sesbania virgata* and velvetleaf can inhibit root elongation of *Arabidopsis*, cress, radish and soybean (Paszkowski and Kremer, 1988; Simões *et al.*, 2008). Weir *et al.* (2006) reported that catechin treatment could cause *Arabidopsis* lipid peroxidation and inhibited plant growth. Catechin may be developed as a powerful herbicide to kill weeds due to its strong phytotoxic effects on roots. However, little is known about precise mode of action of catechin and the response of plant cell membrane lipids to catechin stress.

Membranes, particularly plasma and chloroplast membranes, are sensitive to environmental stimuli, the membrane lipids is crucial in plants response to stresses. PA is an important second messenger, it involves in plant response to various stresses (Testerink and Munnik, 2005; Wang, 2004, 2005b; Wang *et al.*, 2006), and its level increases within minutes under these stresses (Munnik, 2001). PC and PE are related to the membrane stabilization (Welti *et al.*, 2002; Yeagle *et al.*, 1976). We found that

through remodeling of membrane lipids plants respond to frequent alterations between high and low temperatures (Zheng *et al.*, 2011). Phospholipase D (PLD) hydrolyzes phospholipids to generate PA. PLD δ is one of the 12 PLDs in *Arabidopsis*, and it involves in plants stress response and PLD δ increases during stress (Wang, 2005a). Zhang *et al.* (2003) found that PLD δ -null cells displayed increased sensitivity to H₂O₂-induced cell death. PLD δ -mediated hydrolysis of phospholipids plays a positive role in the plant response to oxidative stress. Li *et al.* (2004) found that PLD δ -KO plants exhibited less tolerance to freezing injuries whereas PLD δ -OE plants exhibited more tolerance. PLD δ and PA signaling may involve in the response of plants to drought and salinity (Hong *et al.*, 2010). However, under chemical stresses, like catechin, the change of lipid and the effects of PLD δ during this stress have not previously been described.

Plant lipidomics based on ESI-MS/MS can tell us the 11 classes of lipid changes under certain conditions (Welti *et al.*, 2002). The purpose of this study was to use lipidomics and *Arabidopsis* PLD δ mutant to determine: (1) harmful effects of catechin to *Arabidopsis* root membrane lipid composition and (2) involvement of PLD δ in root membrane lipid profiling.

1 Materials and methods

1.1 Plant materials and chemicals

A PLD δ -knockout (PLD δ -KO) mutant isolated from *Arabidopsis* (Wassilewskija ecotype (WS)) was from Dr. Xuemin Wang's Laboratory previously; the loss of PLD δ was confirmed by the absence of the transcript, protein, and activity of PLD δ (Zhang *et al.*, 2003). (±)-catechin and (+)-cate-

chin were purchased from Sigma-Aldrich (C1788, C1251 respectively).

1.2 Plant growth and treatments

Seeds of two *Arabidopsis* genotypes were sterilized with ethanol (75%) for 2 min and sodium hypochlorite (5%) for 2 min, and then rinsed three times with sterile distilled water. Surface-sterilized seeds were cold stratified for 2 days at 4 °C, and then sowed on 1/2 MS medium (Murashige and Skoog, 1962) for hydroponic culture as described by Tocquin *et al.* (2003). The conditions of the growth chamber were 23/18 °C, a 12/12 h light/dark cycle, and 120 $\mu\text{mol} \cdot \text{m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density.

We used 40 day old hydroponic seedlings to test the effects of catechin on *Arabidopsis* roots. Catechin was added to the hydroponic solutions to the concentration of 150 $\text{mg} \cdot \text{L}^{-1}$, and hold for 0, 10, 30, 90 min. Roots were harvested for lipids analysis at each time.

1.3 Lipid extraction and ESI-MS/MS analysis

The process of lipid extraction, ESI-MS/MS analysis, and quantification was performed as described previously with minor modifications (Devaiah *et al.*, 2006; Welti *et al.*, 2002). Briefly, the roots were cut at sampling time and, to inhibit lipolytic activities, were transferred immediately into 3 mL of isopropanol with 0.01% butylated hydroxytoluene at 75 °C. The roots were extracted with chloroform/methanol (2:1) two additional times with 3 day of agitation each time. The remaining plant roots were heated overnight at 105 °C and weighed. The weights of these extracted and dried tissues were described as “dry weight” of the plants. Lipid samples were analyzed on a triple quadrupole MS/MS equipped for ESI. Data processing was performed as previously described (Devaiah *et al.*, 2006; Welti *et al.*, 2002).

1.4 Data analysis

Statistical analysis was performed using Origin 7.0 (OriginLab Corporation, Northampton, MA, USA). Double bond index (DBI) were calculated with the formula: $\text{DBI} = [\sum (N_1 \times \text{mol\% lipid})]/100$, where

N_1 was the total number of double bonds in the two fatty acid chains of each glycerolipid molecule (Zheng *et al.*, 2011). Average carbon number (C) of acyl chains of lipid classes were calculated by the formula: $C = [\sum (N_2 \times \text{mol\% lipid})]/100$, where N_2 was the total acyl carbons in each lipid molecule.

2 Results and discussion

2.1 Root lipids profiling of *Arabidopsis* during catechin treatment

To test the effects of catechin on *Arabidopsis* root membrane lipids, we used a lipidomics approach to profile changes in molecular species of membrane glycerolipids in *Arabidopsis* during catechin treatment. We identified and quantified about 120 glycerolipids molecular species including 11 species of lipids in *Arabidopsis* roots during catechin treatment (Figs. 1–3). The lipid profile of root is different from that of leaf, where PC and PE are the main lipids of root. The major lipid molecules of MGDG were 34:6MGDG and 36:6MGDG, in roots the content of 36:6MGDG was a bit more than 34:6MGDG, but in leaves the content of 34:6MGDG was much more than 36:6MGDG (Li *et al.*, 2008) (our unpublished data).

To assess the role of PLD δ in *Arabidopsis* response to catechin, we employed the PLD δ knockout mutant *Arabidopsis* and compared its lipid profiles to that of wild type plants during catechin treatment. In wild type (WS) *Arabidopsis* plants, the content of total lipids decreased with the time of catechin treatment. After 90 min treatment with catechin, the content of DGDG, MGDG, PG, PC, and PI decreased both in WS and PLD δ -KO roots, PE and PS decreased in WS but increased in PLD δ -KO plants (Fig. 1). PA is an important secondary messenger in response of plants to abiotic stresses. It can increase in minutes after stimuli and then decreases to the levels of control (Wang *et al.*, 2006). In our experiment, PA rose at the begin of catechin treatment and declined to the level of the control after 90 min treatment in wild plants. While in PLD δ -KO

plants, PA decreased during the catechin treatments (Fig. 1). PLD δ can hydrolyze phospholipids to PA (Zhang *et al.*, 2003), and suppression of PLD δ leads no PA increase during catechin treatment. We thought that PLD δ derived PA was very important for *Arabidopsis* response to catechin stress.

The total content of LysoPLs was a bit different in the two plants. Detailed analysis of the lipid profiles indicated that changes in the phospholipids and galactolipids in the two plants were similar during catechin treatment (Fig. 2, 3), except for minor differences in PE species 34:2, 34:3, and 36:5 (total acyl chains: double bonds), in PA species 34:2, 34:3, and 36:5 (Fig. 2), and in lysophospholipids and lysoPC species 18:2 and 18:3 (Fig. 3).

2.2 PC and PE ratio reduced in PLD δ -KO under catechin stress

PC and PE are related to the stabilization of membrane. Unsaturated PEs have strong propensity to form hexagonal phases (Cullis and Hope, 1978)

which might lead to the formation of a nonbilayer lipid phase and disturb the membrane integrity and cell function, whereas PC is a bilayer-stabilizing lipid (Welti *et al.*, 2002). The molar ratio of PC/PE, which implies the membrane stabilization, tends to drop in plants under cold and hydration stress (Hazei and Williams, 1990; Welti *et al.*, 2002). To assess the membrane stabilization of *Arabidopsis* during catechin treatment, we calculated PC and PE ratio of this process. We found that both PC and PE decreased during catechin treatment in WS, whereas the content of PC decreased and PE increased during catechin treatment in PLD δ -KO plants (Fig. 1). The ratio of PC/PE in WS did not change too much during catechin treatment, however, the PC/PE ratio in PLD δ -KO plants dropped from 1.18 to 0.92 after 90 min of catechin treatment (Table 1). The results suggested that PLD δ play an important role in maintaining membrane stabilization in catechin induced *Arabidopsis* membrane disturbance.

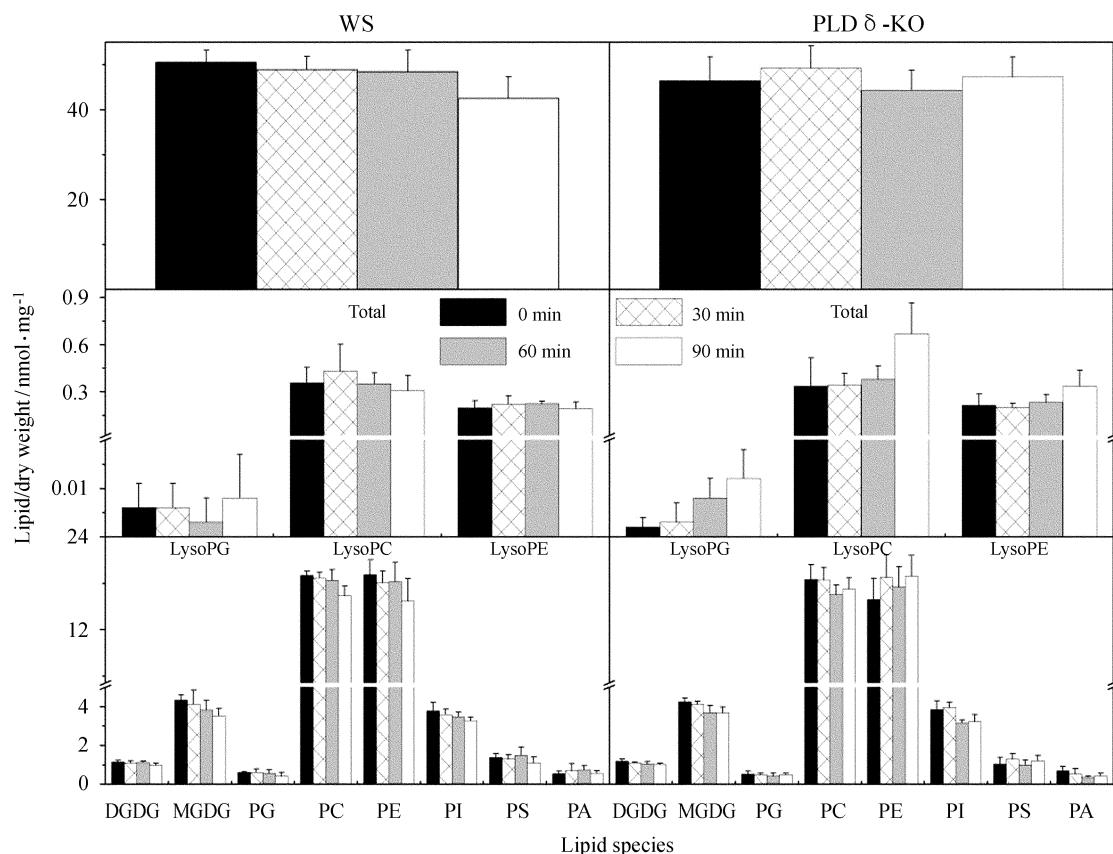


Fig. 1 Changes of each head group class and total lipids in WS and PLD δ -KO plants during catechin treatment. Values are means \pm S. D. ($n=5$)

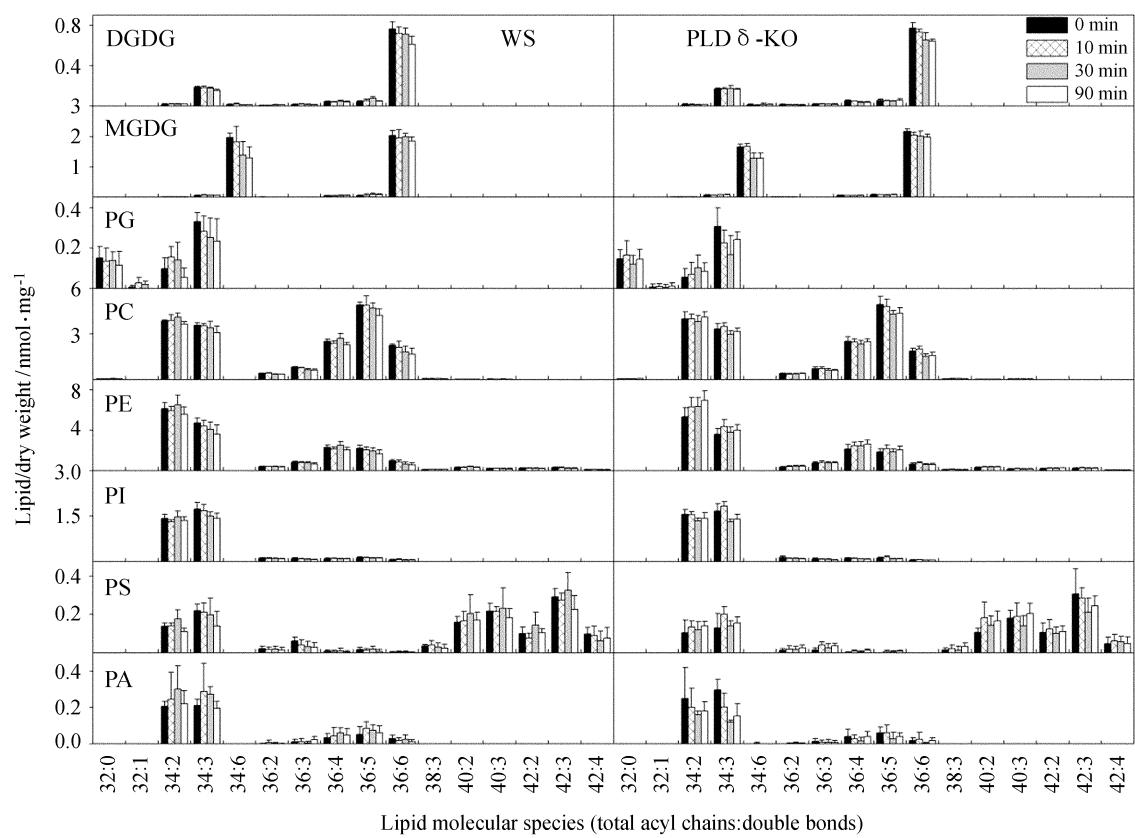


Fig. 2 Changes of the molecular species of membrane lipids in WS and PLD δ -KO plants during different time of catechin treatment. Values are means \pm S. D. ($n=5$)

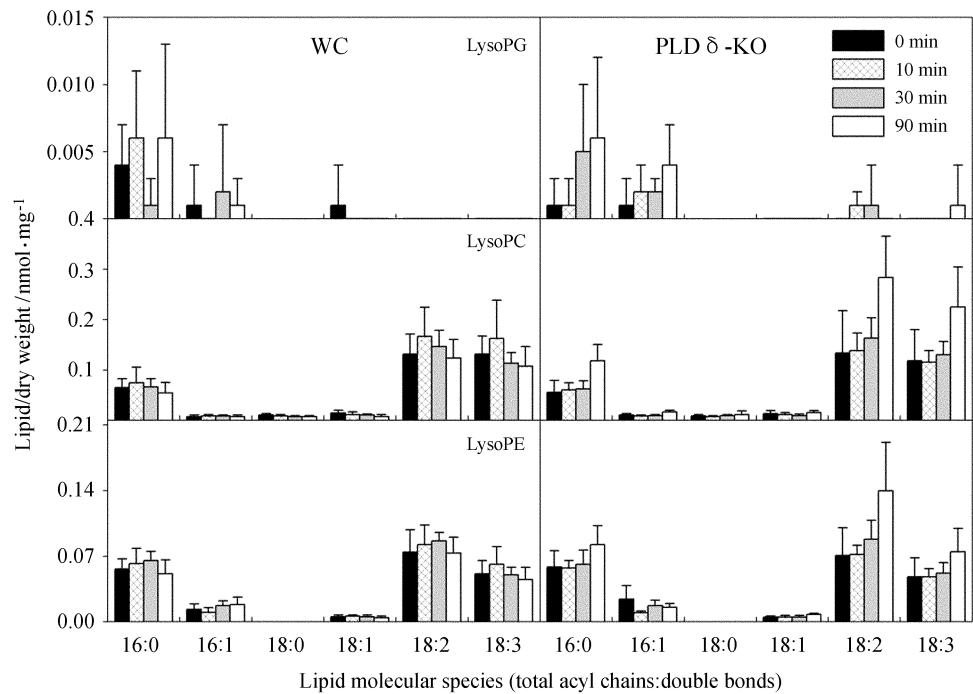


Fig. 3 Changes of the molecular species of lysophospholipids in WS and PLD δ -KO plants during different time of catechin treatment. Values are means \pm S. D. ($n=5$)

Table 1 Lipid ratio of WS and PLD δ -KO plants during catechin treatment. An asterisk indicates that the value is different from control ($P<0.05$). Values are means $\pm SD$ ($n=5$)

Lipids	Plants	Lipid ratio			
		0 min	10 min	30 min	90 min
PC/PE	WS	1.00 \pm 0.07	1.04 \pm 0.06	1.02 \pm 0.09	1.06 \pm 0.13
	PLD δ -KO	1.18 \pm 0.12	0.99 \pm 0.08 *	0.95 \pm 0.09 *	0.92 \pm 0.09 *
(PC+PE)/Total	WS	0.75 \pm 0.01	0.75 \pm 0.02	0.76 \pm 0.01	0.76 \pm 0.02
	PLD δ -KO	0.74 \pm 0.02	0.75 \pm 0.02	0.77 \pm 0.01 *	0.76 \pm 0.02
Glycolipids/Total	WS	0.11 \pm 0.01	0.11 \pm 0.01	0.10 \pm 0.01	0.11 \pm 0.01
	PLD δ -KO	0.12 \pm 0.01	0.11 \pm 0.01	0.11 \pm 0.01	0.10 \pm 0.01 *

2.3 The influence of catechin to lipid double bond index and acyl chain lengths

Maintaining the integrity and optimal fluidity of the membranes is very important to organisms. Changing in the degree of lipid unsaturation and the number lipid acyl chain carbon could influence the integrity and fluidity of membranes. We got the mol% content of each lipid molecule species based on the data of nmol/mg dry weight, and calculated the DBI and Carbon number of acyl chains of each lipid species.

We found that unlike the DBI of leaves, total lipid DBI of roots was about 3.5, but in leaves the DBI was about 4.1 (Zheng *et al.*, 2011) (Table 2), because the main lipid constituent in root are PC and PE which have less double bond than MGDG and DGDG which was the main lipid in leaves. The degree of unsaturation of root membrane lipids is less than leaves, the DBI of PG in roots was about 1.2 less than in roots, the DBI of MGDG, DGDG, and PS was a bit less than that in leaves (Li *et al.*, 2011) (Table 2). The DBI decreased as the catechin

Table 2 Double bond index (DBI) of membrane during catechin treatment in WS and PLD δ -KO plants. An asterisk indicates that the value is different from control ($P<0.05$). Values are means $\pm SD$ ($n=5$)

Lipid species	Plants	Double bond index			
		Control	10 min	30 min	90 min
DGDG	WS	5.14 \pm 0.04	5.12 \pm 0.03	5.08 \pm 0.05	5.06 \pm 0.02 *
	PLD δ -KO	5.13 \pm 0.04	5.15 \pm 0.04	5.08 \pm 0.05	5.08 \pm 0.06
MGDG	WS	5.83 \pm 0.02	5.81 \pm 0.07	5.78 \pm 0.03 *	5.77 \pm 0.05 *
	PLD δ -KO	5.79 \pm 0.02	5.80 \pm 0.01	5.77 \pm 0.02	5.77 \pm 0.02
PG	WS	2.02 \pm 0.23	1.96 \pm 0.31	1.92 \pm 0.26	2.04 \pm 0.17
	PLD δ -KO	2.03 \pm 0.20	1.82 \pm 0.32	1.66 \pm 0.37	1.82 \pm 0.18
PC	WS	3.76 \pm 0.02	3.75 \pm 0.11	3.71 \pm 0.04 *	3.71 \pm 0.07
	PLD δ -KO	3.74 \pm 0.01	3.75 \pm 0.02	3.68 \pm 0.05 *	3.65 \pm 0.02 *
PE	WS	3.14 \pm 0.01	3.12 \pm 0.05	3.05 \pm 0.06 *	3.04 \pm 0.05 *
	PLD δ -KO	3.11 \pm 0.02	3.11 \pm 0.01	3.04 \pm 0.02 *	3.03 \pm 0.02 *
PI	WS	2.69 \pm 0.03	2.71 \pm 0.04	2.66 \pm 0.06	2.67 \pm 0.03
	PLD δ -KO	2.65 \pm 0.01	2.69 \pm 0.03 *	2.64 \pm 0.02	2.63 \pm 0.01 *
PS	WS	2.79 \pm 0.04	2.77 \pm 0.10	2.67 \pm 0.11	2.69 \pm 0.10
	PLD δ -KO	2.71 \pm 0.06	2.69 \pm 0.08	2.68 \pm 0.04	2.67 \pm 0.04
PA	WS	3.02 \pm 0.16	3.06 \pm 0.17	2.99 \pm 0.13	2.99 \pm 0.16
	PLD δ -KO	2.97 \pm 0.07	3.01 \pm 0.22	2.75 \pm 0.25	2.98 \pm 0.09
Total Lipids	WS	3.58 \pm 0.01	3.56 \pm 0.08	3.49 \pm 0.07	3.51 \pm 0.05
	PLD δ -KO	3.58 \pm 0.03	3.55 \pm 0.02	3.49 \pm 0.03 *	3.44 \pm 0.04 *

treatment, and there was no difference in DBI between WS and PLD δ -KO plants except for PG, which DBI in PLD δ -KO plants was less than in WS when treated with catechin (Table 2). These results suggested that catechin may induce membrane lipids oxidation.

In the eight classes of lipids that calculated acyl chain carbons, the acyl chain carbons of MGDG of roots was longer than that in leaves (Table 3, our

unpublished data). The longest acyl chain was PS which was about 39 carbons (Table 3). Under catechin treatment, the acyl chain carbons of the lipids change little except that of PS which in WS increased 0.4 and in PLD δ -KO decreased 0.66 after 90 min of catechin treatment. Catechin could induce change of length of lipid acyl chain, the most sensitive lipid is PS.

Table 3 The acyl chain carbon of membrane lipids during catechin treatment in WS and PLD δ -KO plants. An asterisk indicates that the value is different from control ($P<0.05$). Values are means $\pm SD$ ($n=5$)

Lipid species	Plants	Acyl chain carbon (C)			
		Control	10 min	30 min	90 min
DGDG	WS	35.54 \pm 0.02	35.53 \pm 0.02	35.56 \pm 0.02	35.54 \pm 0.03
	PLD δ -KO	35.59 \pm 0.02	35.59 \pm 0.02	35.53 \pm 0.03 *	35.53 \pm 0.03 *
MGDG	WS	35.00 \pm 0.03	35.03 \pm 0.09	35.16 \pm 0.11 *	35.15 \pm 0.11 *
	PLD δ -KO	35.10 \pm 0.01	35.08 \pm 0.02	35.18 \pm 0.04 *	35.17 \pm 0.04 *
PG	WS	33.47 \pm 0.20	33.46 \pm 0.22	33.40 \pm 0.17	33.47 \pm 0.14
	PLD δ -KO	33.42 \pm 0.09	33.31 \pm 0.23	33.31 \pm 0.25	33.37 \pm 0.19
PC	WS	35.20 \pm 0.02	35.19 \pm 0.05	35.17 \pm 0.01 *	35.16 \pm 0.02 *
	PLD δ -KO	35.20 \pm 0.01	35.18 \pm 0.01 *	35.17 \pm 0.03	35.14 \pm 0.02 *
PE	WS	35.22 \pm 0.02	35.21 \pm 0.04	35.16 \pm 0.05 *	35.16 \pm 0.03 *
	PLD δ -KO	35.22 \pm 0.02	35.20 \pm 0.02	35.16 \pm 0.01 *	35.15 \pm 0.01 *
PI	WS	34.29 \pm 0.03	34.29 \pm 0.02	34.26 \pm 0.01	34.27 \pm 0.02
	PLD δ -KO	34.29 \pm 0.03	34.26 \pm 0.03	34.28 \pm 0.02	34.24 \pm 0.03 *
PS	WS	38.76 \pm 0.12	38.75 \pm 0.18	38.88 \pm 0.45	39.16 \pm 0.38
	PLD δ -KO	39.48 \pm 0.46	38.89 \pm 0.13 *	38.93 \pm 0.55	38.82 \pm 0.31 *
PA	WS	34.45 \pm 0.17	34.50 \pm 0.10	34.45 \pm 0.07	34.53 \pm 0.11
	PLD δ -KO	34.36 \pm 0.15	34.43 \pm 0.13	34.33 \pm 0.20	34.47 \pm 0.13
Total Lipids	WS	35.20 \pm 0.02	35.19 \pm 0.04	35.19 \pm 0.05	35.18 \pm 0.03
	PLD δ -KO	35.19 \pm 0.02	35.19 \pm 0.03	35.17 \pm 0.04	35.16 \pm 0.02 *

In conclusion, the results of this study suggested that PLD δ deficient plant was more sensitive to catechin stress than wild plant. Catechin could induce the root lipids change and lead to the membrane disturbance. However, many questions, such as the mechanisms of different lipid profiles in roots and leaves; the role of PLD δ play in *Arabidopsis* resist to catechin stress; and the reason of changes of acyl chain carbon length to catechin stress, remained unclear and would be further studied in the future.

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